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Review

A review of vaccine research and development: Human acute respiratory infections☆

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Abstract

Worldwide, acute respiratory infections (ARIs) constitute the leading cause of acute illnesses, being responsible for nearly 4 million deaths every year, mostly in young children and infants in developing countries. The main infectious agents responsible for ARIs include influenza virus, respiratory syncytial virus (RSV), parainfluenza virus type 3 (PIV-3), *Streptococcus pneumoniae* and *Haemophilus influenzae*. While effective vaccines against influenza, *H. influenzae* type b (Hib) and *S. pneumoniae* infections have been available for several years, no vaccine is available at present against illnesses caused by RSV, PIV-3, metapneumovirus or any of the three novel coronaviruses. In addition, the threat constituted by the multiple outbreaks of avian influenza during the last few years is urgently calling for the development of new influenza vaccines with broader spectrum of efficacy, which could provide immunity against an avian influenza virus pandemic. This article reviews the state of the art in vaccine R&D against ARIs and attempts to address these basic public health questions.

Keywords: Vaccines; SARS; Influenza; Pneumococcal infection; *Streptococcus pneumoniae*; *Haemophilus influenzae*; Hib; Respiratory Syncitial Virus; RSV; Parainfluenza viruses; PIV; Bronchiolitis

Contents

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1. Introduction

Acute respiratory infections (ARI) continue to be the leading cause of acute illnesses worldwide. Whereas upper respiratory infections (URIs) are very common but seldom life-threatening, lower respiratory infections (LRIs) are responsible for more severe illnesses such as pneumonia and bronchiolitis and are the leading contributors to the nearly 4 million deaths caused each year by respiratory diseases [1]. Most of these deaths occur in children in developing countries, particularly those living in Africa and Southeast Asia [2]. The populations at greatest risk for developing a fatal respiratory infection are the very young, the elderly, and the immunocompromised. The overlaps in the clinical manifestations of the different syndromes that constitute LRIs, the difficulties in establishing precise bacteriological or viral etiology, and the frequent occurrence of concurrent infection with more than one pathogen, including measles, make the estimation of syndrome-specific or pathogen-specific disease burden particularly difficult. However, the substantial reduction in LRI mortality observed in studies in several developing countries that have implemented simple case management based on early detection and provision of antibiotics to children with LRI suggests that bacterial pneumonia contributes to the majority of deaths in these populations [3]. In addition, nosocomial or hospital-acquired pneumonia is a major infectious problem: pneumonia is the second most common type of nosocomial infections, with an associated case fatality rate of 20–50%.

The main etiological agents responsible for ARIs in children include *Streptococcus pneumoniae*, *Haemophilus influenzae*, respiratory syncytial virus (RSV), and parainfluenza virus type 3 (PIV-3). In the elderly, influenza-related pneumonia remains a leading cause of infectious disease-related deaths. Several novel agents associated with LRIs were discovered in the past 4 years including metapneumovirus [4,5], the severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV) [6,7] and human pneumonia-associated coronaviruses NL63 [8,9] and HKU1 [10]. Measles, adenovirus and rhinoviruses also are a frequent cause of respiratory diseases. In addition, members of the herpesvirus family, including herpes simplex, cytomegalovirus, varicella-zoster virus, Epstein-Barr virus and human herpes virus type 6 (HHV6) also have been associated with respiratory disease [11]. It has been estimated that one in five hospitalizations that occur each year in the USA for LRI in children less than 18 years of age are due to RSV, 12% to PIV-1, -2, and -3, and 7% to influenza virus [12].

Available data also suggest that dual infection with viruses and bacterial pathogens are quite common, especially in developing countries. In a study in Papua New Guinea, bacteria were isolated from blood culture or lung aspirate in two-thirds of children with viral ARI [13]. More recently, an efficacy trial of a 9-valent pneumococcal vaccine in South Africa showed 31% reduction in hospital virus-associated pneumonia in the vaccinated group, suggesting that these children had dual infection [14]. Although the exact relationship between viral and bacterial infection in these cases has not been established, dual infection seems to increase the severity of the disease and to result in higher mortality.

Today, the looming new threat seems to be avian influenza. The multiple independent outbreaks of the last few years,

involving several different virus strains in Asia, North America and the Netherlands, are dire warnings of a potential flu pandemic. It is impossible to predict which virus is going to cause it and when it is going to happen [15], but preparation for this potential event is going on in many parts of the world [16–21]. Using the techniques of reverse genetics, it is now possible to develop a vaccine against any potential pandemic influenza virus strain such as the avian H5N1 strain. Moreover, a number of influenza subunit or DNA vaccine candidates are in various stages of development, which bears hope for inducing broad spectrum immunity against multiple strains of influenza virus.

Live, attenuated parainfluenza virus (PIV) vaccines are being developed for delivery by the intranasal route from both human strains and bovine isolates, which are closely related antigenically to human PIV-3. The first vaccine candidates were found to be over-attenuated, which has prompted work on chimeric bovine/human PIV-3 candidate vaccine. Similarly, development of live, attenuated RSV vaccines that could be delivered intranasally has met with the difficulties of over- or under-attenuation of the virus and limited genetic stability. A number of subunit vaccines – based on the F, G and M proteins from RSV – are in development, keeping in mind that studies in the 1960s have shown that children vaccinated with an inactivated RSV vaccine suffered from more severe disease on subsequent exposure to the virus.

A large number of candidate vaccines against SARS also are being developed, including whole inactivated virus vaccine preparations, live virus-vectored recombinant vaccines expressing SARS-CoV proteins, DNA vaccines, as well as subunit vaccines. All these vaccines, however, face uncertainties, due to the lack of a reliable animal model in which to test them and to the uncertainty on the occurrence of new outbreaks of the disease.

Protective immunity against *S. pneumoniae*-induced illnesses is conferred by type-specific, anticapsular polysaccharide (PS) antibodies. Several vaccine manufacturers have developed pneumococcal conjugate vaccines in which *S. pneumoniae* PS are covalently coupled to a protein carrier. Introduction of a pneumococcus conjugate vaccine in early 2000 in the USA resulted in dramatic decline in the rates of invasive pneumococcal disease. Unfortunately, the currently licensed 7-valent conjugate vaccine does not contain some of the serotypes that cause severe disease in developing countries. The recently published results of a Phase III clinical trial with a 9-valent vaccine in The Gambia showed 77% efficacy against vaccine type invasive disease and 16% reduction in mortality in the vaccinated group [14], which gives hopes that introduction of pneumococcus conjugate vaccines will have a dramatic effect on child survival in developing countries. Newer approaches based on pneumococcal proteins are at preclinical or early clinical stages of development.

Conjugate vaccines against *Haemophilus influenza* type b are highly effective in preventing Hib induced disease. Unfortunately, the vaccine remains expensive for the world's poorer nations, which would benefit the most from introducing it into their national immunization system. The Global Alliance for Vaccines and Immunization (GAVI) has been offering support for Hib vaccine introduction in developing countries since 2000.

This article will review ongoing development of future vaccines to combat these various illnesses.

2. Haemophilus influenzae type b

2.1. Disease burden

Haemophilus influenzae type b, or Hib, is currently responsible for some 3 million serious illnesses and nearly 400,000 deaths per year, chiefly through meningitis and pneumonia. Almost all victims are children less than 5 years of age. Conventional studies on the burden of Hib disease have focused on meningitis and invasive disease. These studies have shown annual incidence rates of laboratory-confirmed Hib meningitis between 20 and 60 cases per 100,000 children aged less than 5 years in industrialized countries. Few prospective, population-based studies have been conducted in countries classified as developing or least developed. In sub-Saharan African, the Middle East, Latin America, and the Pacific Islands including the Philippines, incidence rates of Hib meningitis are comparable to or higher than in North America and Europe. In contrast, incidence rates were found to be substantially lower in Asia and Eastern Europe. However, as these studies relied on microbiological identification of Hib, they may have underestimated the actual disease burden [1,22].

The burden of Hib pneumonia is most likely higher than that of invasive disease, but it remains difficult to define since existing techniques to establish bacterial etiology lack sensitivity and specificity. A review of several studies from Asia, Africa and Oceania conducted since the 1980s show that *S. pneumoniae* and *H. influenzae* account for 13–34% and 1.4–42% of bacterial pneumonia, respectively [23]. In several studies in which serotyping was performed, type b accounted for 5–11% of pneumonia cases [13,24,25]. Field trials with the Hib conjugate vaccine have demonstrated a 20% reduction in pneumonia following vaccination in The Gambia [26] and a 21% reduction in hospitalized pneumonia in Chile [27]. A recent estimate from Brazil showed a 31% reduction in radiological pneumonia following Hib vaccination [28]. On the other hand, a prospective randomized controlled trial of Hib vaccine in Lombock, Indonesia, found no reduction in radiological pneumonia and only a 2% reduction in clinical pneumonia, suggesting low frequency of Hib pneumonia in that part of Asia [22].

2.2. Bacteriology

H. influenzae is a small fastidious bacterium that requires hemin (X factor) and nicotinamide adenine dinucleotide (NAD or V factor) for growth. This explains why it does

not grow on regular blood agar but only on chocolate agar, made by heating blood so that red blood cells lyse and release their V factor. Based on differences in the composition of the polysaccharide capsule, six serotypes have been identified (a–f). Serotype b (Hib) is the cause of over 90% of all invasive infections. In addition, the bacteria can be classified into six different biotypes (I–VI). Non-encapsulated strains of *H. influenzae* seldom cause invasive disease but are an important cause of pneumonia.

2.3. Vaccines

Currently, three conjugate Hib vaccines exist that differ by the nature of the carrier protein, the size and amount of the capsular PS and the conjugation method. These are: HbOC, whose carrier is mutant diphtheria toxin CRM197; PRP-T, which uses the tetanus toxoid as a carrier; and PRP-OMP, whose carrier is the meningococcal outer membrane protein. Although the dynamics of the immune response elicited by each vaccine may vary, the responses following a primary series of vaccination are comparable [29,30]. The efficacy of these vaccines to prevent not only meningitis but also pneumonia has been well documented in several studies in industrialized countries as well as in The Gambia [26,31,32]. All studies demonstrated greater than 90% protective efficacy against laboratory-confirmed invasive disease, irrespective of the choice of the vaccine. Consequently, Hib vaccination was included in the national immunization programme of industrialized countries, which resulted in the near-elimination of invasive Hib disease, thanks to the fact that mucosal immunity in the vaccinees resulted in decreased transmission of the pathogen in the population [32,33]. Available data in a few developing countries also show a similar herd immunity effect [34,35]. The possibility of vaccinating newborns through maternal immunization also has been studied [36].

Although Hib vaccines are highly effective vaccines, and despite the success of the vaccine in industrialized countries, its cost unfortunately remains too high for the world's poorest nations (the vaccine costs nearly seven times the total cost of all other routine childhood vaccines together) and its sustained use is threatened in the few developing countries that have introduced it in their immunization programme. Definite progress has been made in the introduction of Hib vaccination in developing countries with the establishment of the Global Alliance for Vaccines and Immunization (GAVI), although significant hurdles still remain. In 2004, a Hib Vaccine Task Force commissioned by GAVI concluded that, in most countries, decision not to introduce the Hib vaccine lacked careful analysis and review of the local burden of the disease and accurate cost:benefit analysis of Hib vaccine introduction. Subsequently, GAVI approved the establishment of a Hib Initiative to support those countries wishing to sustain Hib vaccine immunization as well as those countries wishing to explore whether the introduction of the vaccine was to be considered a priority in their immunization programme.

3. Influenza

3.1. Disease burden

The burden of influenza in the USA is currently estimated to be 25–50 million cases per year, leading to 150,000 hospitalizations and 30,000–40,000 deaths. If these figures are extrapolated to the rest of the world, the average global burden of inter-pandemic influenza may be on the order of ~1 billion cases, including ~3–5 million cases of severe illness and 300,000–500,000 deaths annually.

Epidemics and outbreaks of influenza occur in different seasonal patterns depending on the region in the world. In temperate climate zones, seasonal epidemics typically begin in the late fall and peak in mid- to late winter. In tropical zones, seasonal patterns appear to be less pronounced, with year-round isolation of virus. In industrialized countries, annual influenza epidemics infect about 5–15% of the population each season, and cause febrile illnesses that range in severity from mild to debilitating and can lead in some instances to hospitalizations and even cause deaths. The latter mostly occur as a consequence of primary fulminant influenza virus pneumonia or of secondary respiratory bacterial infections and are facilitated by underlying pulmonary or cardiopulmonary pathologies. The risk of developing serious complications is aggravated in the very young and in the elderly. Data collected in Michigan (USA) and in Japan indicate that mass vaccination of school-aged children correlates with a reduced rate of respiratory illness in all age groups, suggesting that large-scale immunization in childhood could favorably affect influenza epidemics.

The repetitive occurrence of yearly influenza epidemics is maintained through the ongoing process of "*antigenic drift*", which results from the accumulation of point mutations in the genes that encode the two viral surface proteins hemagglutinin (HA) and neuraminidase (NA), and leads to the constant emergence of new virus strains against which there is little or no pre-existing immunity in the population [37,38]. At unpredictable intervals, due to the segmented nature of the influenza virus genome, these viruses also can acquire new genes from an avian or other animal influenza virus. This process is believed to occur most readily in pigs, as these animals can be infected by avian as well as human viruses. Co-infection in pigs can result in the emergence of a virus with a completely new glycoprotein subtype, an event which is referred to as an "*antigenic shift*". If the recombined virus infects the human population and succeeds in efficiently spreading from person-to-person, a worldwide epidemic known as a pandemic can occur [19]. Three of these pandemics occurred in the last century (1918, 1957, and 1968). The most severe, in 1918, infected approximately 50% of the world's population, killing an impressive 20–50 million people, particularly those in the prime of their lives. This pandemic depressed population growth for the following 10 years [39].

One of the last outbreak of influenza with high mortality and pandemic potential occurred in 1997, when a new influenza virus with an avian virus HA glycoprotein (H5N1) emerged in Hong Kong, China, killing 6 of the 18 affected patients, mainly young adults. Fortunately, the virus was not able to spread from person-to-person and it was possible to stop the outbreak by massive culling of poultry. Another H5N1 strain was isolated in 2003–2004 in several countries in Asia, including Vietnam and Thailand, killing altogether 31 of the 43 patients diagnosed with the virus [15,40]. Again, from 16 December 2004 to 15 May 2005, 49 new cases of H5N1 influenza were reported from Vietnam, including 17 deaths, and 4 lethal cases from Cambodia. Seventeen months into the current outbreak, the global official number of human victims of the H5N1 virus has passed the 70 mark.

These recent outbreaks have coincided with a major epizootic of avian flu in South East Asia, due to a highly pathogenic H5N1 virus strain that not only kills domestic poultry (except ducks) but also wild birds such as geese, flamingos, and other species of aquatic birds [41]. The virus is also pathogenic for ferrets, cats and tigers. Cats can be infected with H5N1 virus both by the respiratory route and by feeding on virus-infected birds. The fear is that the H5N1 virus could gain the capacity to spread into the human population through change in receptor-binding specificity by mutation or reassortment, leading to a new pandemic. So far, no human-to-human transmission has been documented with certitude, although the increasing number of reported family clusters of H5N1 in Vietnam might signal human-to-human transmission. The finding that several poultry workers carried antibodies to the H5NI virus after the 1997 outbreak in Hong Kong as well as after the 2003–2004 outbreak in Japan, point to the existence of mild or asymptomatic avian flu infections in humans, but a concerted action to establish the true extent of the spread of the virus still is lacking. Meanwhile, fresh bird outbreaks of H5N1 have been reported in Indonesia and the fear is that North Korea might be infected as well [42].

Other avian influenza viruses have occasionally caused a human outbreak, such as a H9N2 strain in 1999 in Hong Kong, H7N7 strain in 2003 in the Netherlands, which caused 89 confirmed human cases with conjunctivitis and one death, and H7N2 and H7N3 strains in 2003–2004 in North America [19].

In the USA, the impact of a new pandemic, assuming it would be of a similar magnitude as the 1957 or the 1968 pandemics, and not like the 1918 pandemic, is projected to be 18–42 million outpatient visits, 314,000–734,000 hospitalizations and 89,000–207,000 deaths [43]. Extrapolating these figures to the world population, a gross estimate of the impact of the next pandemic calls for 1–2 billion cases of flu, 5–12.5 million cases of severe illness, and 1.5–3.5 million deaths worldwide! The economic cost of a 1968-style pandemic has been estimated at about US$ 167 billion for all industrialized countries combined, including only direct medical costs and lost productivity as a result of disease and deaths [16].

3.2. Virology

Influenza viruses are enveloped viruses with a segmented genome made of eight single-stranded negative RNA segments, most of which encode only one viral protein (e.g. HA, NA, M, NP). Influenza viruses belong to the family *Orthomyxoviridae* [38]. They are divided into three genera, Influenzavirus A, Influenzavirus B, and Influenzavirus C, based on antigenic differences in two of their structural proteins, the matrix protein (M) and the nucleoprotein (NP). Influenza A viruses are further divided into subtypes according to the antigenicity of their major envelope glycoproteins, HA and NA. Fifteen HA subtypes (H1–H15) and nine NA subtypes (N1–N9) have been identified so far. Only viruses of the H1N1, H1N2 and H3N2 subtypes are currently circulating in the human population.

Influenza A viruses also infect poultry, aquatic birds, pigs, horses, and sea mammals. Aquatic birds, in which the virus multiplies in the gut, usually have an asymptomatic infection and excrete the virus in their feces, thus serving as a natural virus reservoir and a potential source of new genes for pandemic influenza viruses. Swapping of genomic segments leading to the emergence of a new, reassortant progeny strain with a mixed genotype most readily occurs in pigs, as pigs have the complete set of sialylated receptors for avian, swine and human influenza virus strains.

HA is present at the surface of the flu virion in the form of a HA0 precursor which must undergo proteolytic cleavage to generate functional subunits HA1, which bears the receptor-binding site and neutralization epitopes, and HA2, which is responsible for the fusion of the viral envelope with the host-cell membrane. After binding of the virion to sialic acid-containing cell receptors, the virus–receptor complex is endocytosed into an endosome whose acidification triggers a conformational rearrangement of viral HA, and the fusion peptide at the N terminus of the HA2 polypeptide is inserted into the target cell membrane. HA2 then folds into a coiled coil, bringing viral and cell membrane in close contact so that fusion can occur [44,46]. Classical avian virus strains have a HA0 trypsin-like cleavage site, hence their tropism for the gastrointestinal tract. In contrast, highly virulent avian strains such as the 2004 H5N1 strain from Vietnam and Thailand have acquired through spontaneous mutations an ubiquitous furin-like cleavage site, which allows them to multiply in many tissues including the respiratory tract.

NA is a neuraminidase that cleaves sialic acid residues, promotes the release of mature virus particles from infected cells and facilitates their dispersion through mucus layers.

Influenza antiviral drugs include amantadine and rimantadine, which are devoid of effect against H5NI virus strains, and zanamivir (Renza) and osaltamivir (Tamiflu), which block the egress of progeny virus particles from infected cells by inhibiting viral neuraminidase activity. These are active on H5NI viruses. These drugs, however, are only available in limited supply [47].

3.3. Vaccines

3.3.1. Inactivated vaccines

The currently available influenza vaccines are made from inactivated, detergent-split influenza virus grown in the allantoic cavity of embryonated chicken eggs. These vaccines effectively prevent influenza-related illness and have a high benefit-to-cost ratio in terms of preventing hospitalizations and deaths, as shown in numerous studies on vaccination of the elderly and of individuals at high risk for severe outcomes of influenza [48]. WHO estimates that there globally are about 1.2 billion people at high risk for severe influenza outcomes: 385 million elderly over 65 years of age, 140 million infants, and several hundred million children and adults with underlying chronic health problem. The last number still remains unknown, although it has been estimated in industrialized countries that around 12% of total population have chronic underlying respiratory diseases, including asthma. In addition, 24 million health care workers should be immunized to prevent them from spreading the disease to the high-risk population. At this time, the world's total vaccine production capacity is limited to about 900 million doses, which realistically does not suffice to cover the global high-risk population.

It is, therefore, quite evident that the global infrastructure would not be able to handle the timely manufacture, distribution and delivery of a pandemic influenza vaccine, which, in all likelihood, would have to be given as a two-dose regimen because people will not have had a previous exposure to the virus antigen [20]. One solution to this problem would be to lower the quantity of antigen per dose and add an adjuvant to the vaccine, but this needs to be tested in clinical trials [49]. The use of an ISCOM (immunostimulating complexes) vaccine accelerated antibody responses in humans and this coincided with proliferative T-cell responses [50].

Another solution would be to improve on current vaccine production technologies (egg-derived vaccines). Several pharmaceutical companies have embarked on projects for the development of cell-culture vaccines, as this could help overcome current vaccine production bottlenecks, limited availability of specific pathogen-free egg supply and time constraints. Furthermore, it would improve possibilities of up-scaling vaccine production capacities in face of a pandemic. Influenza virus can be adapted to grow on a variety of mammalian cell lines, including Vero, PER.C-6, and Madin-Darby canine kidney (MDCK) cell lines. Crucell (Holland) is partnering with Sanofi-Pasteur (France) to develop large-scale cell-culture methods using human PER.C-6 cells for both a pandemic vaccine and the annual influenza vaccine. Clinical trials are expected to start in the summer of 2005.

It is now possible to develop a vaccine against any potential pandemic influenza virus strain such as the avian H5N1 strain, using the techniques of reverse genetics [51]. This process requires to first mutagenize the HA1/HA2 cleavage site of the new strain so as to attenuate its virulence [52], then to transfer the HA and NA genomic segments into an appropriate influenza A virus master strain such as the PR8 strain which has been adapted to grow on Vero cells, thus generating within a few weeks a reassortant virus with the antigenic specificity of the pandemic strain and the growth characteristics of the master strain, including adaptation to cell culture [53,54].

To assess dosage for the reverse-genetics vaccine against Vietnam H5N1 virus, clinical trials are being conducted by the NIH, Bethesda, USA using chicken egg-grown vaccine lots prepared by Chiron and by Sanofi-Pasteur. Other countries are following on that path and will soon embark on testing the immunogenicity of low dose or alum-adjuvanted H5N1 vaccine. Intellectual property and liability issues are considered as obstacles to the industrial development of reverse-genetics-based vaccines, not counting the fact that these viruses are considered genetically modified organisms and as such will need special clearance in some countries [17].

Other formulations of inactivated influenza vaccine for mucosal delivery are being developed, including an ISCOMATRIX adjuvanted vaccine [55] and a powder vaccine that is developed against the avian H5N1 virus strain by Texas-based DelSite Biotechnologies [56].

It is to be noted that an inactivated vaccine against the Asia 2003-H5N1 virus strain has been developed in veterinary medicine using the H5N9 (A/Turkey/Wisconsin/1968) avian influenzavirus strain that is less pathogenic, grows to high titers in embryonated chicken eggs and, once inactivated, provides 100% protection against H5N1 virus challenge in poultry.

3.3.2. Live attenuated vaccines

Another approach to influenza vaccines has been the development of cold-adapted (*ca*) virus strains which grow well in primary chick kidney cells and embryonated eggs at 25–33 °C, have a reduced replication titer at 37 °C, and show attenuated behavior in ferrets. Cold adaptation was found to be a reliable and efficient procedure for the derivation of live attenuated influenza virus vaccines for humans. The process of genetic reassortment with the transfer of the six internal genes from a stable attenuated *ca* master donor strain of influenza A virus or influenza B virus to the new prevailing wild-type epidemic strain has been used to generate attenuated cold-reassortant vaccines with the proper level of attenuation and immunogenicity in humans, genetic stability, and low or absent transmissibility from vaccinees to contacts [57]. A trivalent live *ca* vaccine (Flumist) has been developed along these lines by MedImmune and Wyeth for intranasal spray delivery.

This vaccine was proven highly efficacious in Phase III trials, showing a 92% overall protection rate over a 2-year study in children, including protection against antigenic variants that circulated in the region in the second year [58]. It efficacy in children over 2 years of age was indeed found superior to that of the inactivated vaccines. The vaccine has been licensed in the USA for vaccination of persons from 5 to 49 years of age, in view of side effects in younger children (wheezing, nasal congestion) and absence of data in the

elderly. The vaccine is safe, effective, and shows remarkable genetic stability, but it has to be kept at −18 °C. A new, heat-stable version of the vaccine has recently been developed, and has shown good efficacy in clinical trials in Asia and Europe, including on young children. Application for European licensure is pending (MedImmune).

Another cold-adapted master virus strain was developed at the Institute of Applied Microbiology (Austria) by growth of wild influenza virus in Vero cells at 25 °C. The live vaccine based on this master virus strain was safe, well tolerated and immunogenic after intranasal immunization in young adult volunteers [59].

Biodiem Limited (Australia) and Merck are developing yet another live attenuated influenza vaccine to be delivered by nasal spray, as is the Vector Scientific Center in Russia, which is focusing on a cold-adapted live attenuated virus grown in MDCK cells on microcarrier beads in serum-free medium. The vaccine is at an advanced preclinical development stage [60].

3.3.3. Subunit and DNA vaccines

A number of influenza subunit or DNA vaccine candidates are in various stages of development.

Berna Biotech is commercializing an influenza vaccine formulated in virosomes, with the surface spikes of the three currently circulating influenza virus strains inserted in the vesicle membrane of three corresponding virosome types [61]. A nasal formulation of this vaccine was, however, recently withdrawn from the market, due to undesirable neurological side effects (Bell's palsy) linked to the presence of the *E. coli* labile toxin (LT) used as an adjuvant, most likely as a consequence of binding of the B subunit of LT to GM1 ganglioside in neuronal tissues associated with the olfactory tract.

Protein Sciences Corp (Connecticut) is developing a subunit vaccine using recombinant HA protein produced in a serum-free insect cell cultures with a baculovirus vector. The vaccine has been successfully tested in a Phase II trial in 64 to 89-year-old volunteers in whom it induced good anti-HA antibody responses. A Phase II/III trial in 460 volunteers is underway (personal communication).

Yeda and BionVax, both Israeli R&D companies, are developing a synthetic peptide influenza vaccine for nasal administration. The vaccine has shown protective efficacy in humanized mice and is planned to enter clinical trials in 2005.

An epidermal DNA influenza vaccine was developed and tested in humans by PowderJect. The vaccine was found to be safe and immunogenic in subjects with prevaccination antibodies to influenza virus (Drape, Macklin et al., in press). Other DNA vaccines for influenza are still at an early stage of development [62], due to poor immunogenicity results of naked DNA in humans.

Finally, a recombinant particulate vaccine has been engineered by genetically fusing copies of the influenza virus transmembrane (M2) protein to the hepatitis B core antigen (HBc). The (M2)-HBc fusion protein spontaneously assembled into virus-like particles (VLP) that provided complete protection against a potentially lethal influenza virus A challenge in mice [63,64]. Similarly, a M2 peptide was conjugated with *Neisseria meningitidis* outer membrane protein complex (OMPC) and recently evaluated in animal models including monkeys [65]. M2 is a highly conserved transmembrane protein in the virion. These approaches might thus serve as a basis for an universal influenza vaccine with broad spectrum of protective activity [66].

4. Parainfluenza viruses

4.1. Disease burden

Parainfluenza viruses cause a spectrum of respiratory illnesses, from URIs, 30–50% of which are complicated by otitis media, to LRIs, about 0.3% of which require hospitalization. Most children are infected by parainfluenza virus type 3 (PIV-3) by the age of 2 years and by parainfluenza virus types 1 and 2 (PIV-1 and -2) by the age of 5 years. PIV-3 infections are second only to RSV infections as a viral cause of serious ARI in young children. Pneumonia and bronchiolitis from PIV-3 infection occur primarily in the first six months of life, as is the case for RSV infection. Croup is the signature clinical manifestation of infection with parainfluenza viruses, especially PIV-1, and is the chief cause of hospitalization from parainfluenza infections in children 2–6 years of age. However, this syndrome is relatively less frequent in developing countries. The proportions of hospitalizations associated with PIV infection vary widely in hospital-based studies [67]. Consequently, the annual estimated rates of hospitalization fall within a broad range: PIV-1 is estimated to account for 5800–28,900 annual hospitalizations in the USA, PIV-2 for 1800–15,600 hospitalizations, and PIV-3 for 8700–52,000 hospitalizations. Along with RSV, parainfluenza viruses are also leading causes of hospitalization in adults with community-acquired respiratory disease.

The seasonal patterns of PIV-1, -2, and -3 infections are curiously interactive. PIV-1 causes the largest, most defined outbreaks, marked by sharp biennial rises in cases of croup in the autumn of odd-numbered years. Outbreaks of infection with PIV-2, though more erratic, usually follow type 1 outbreaks. Outbreaks of PIV-3 infections occur yearly, mainly in spring and summer, and last longer than outbreaks of types 1 and 2. Although PIV-1 to -3 have been described as a cause of LRI in developing countries, the disease burden has not been accurately quantified in these countries.

4.2. Virology

Parainfluenza viruses belong to the family *Paramyxoviridae*, subfamily *Paramyxovirinae*, itself subdivided into three genera: Paramyxovirus (PIV-1, PIV-3, and Sendai virus), Rubulavirus (PIV-2, PIV-4 and mumps virus) and Morbillivirus (measles virus). All are enveloped viruses with

a negative strand, ~15,500 nucleotide-long nonsegmented RNA genome which encodes two envelope glycoproteins, the hemagglutinin-neuraminidase (HN), and the fusion protein (F, itself cleaved into fusion active disulfide-linked F1 and F2 subunits), a matrix protein (M), a nucleocapsid protein (N) and several nonstructural proteins including the viral replicase (L).

The HN protein mediates primary attachment of the virion to target cells while the F protein promotes fusion between the virus envelope and the cell membrane. Membrane fusion is initiated by the insertion into the cell membrane of the viral fusion peptide, located at the N terminus of the F1 subunit, while the two heptad repeat regions in F1, one immediately C-terminal to the fusion peptide and the other adjacent to the transmembrane domain, fold into trimeric helical coiled-coils (six-helix bundles), which brings the virus envelope into close contact with the cell membrane [68]. A similar mechanism has been reported for a number of viral fusion proteins, including that of other paramyxoviruses (RSV), the HIV Env glycoprotein (gp41) and the low-pH form of the influenza virus HA protein (for reviews, see [44,45]).

4.3. Vaccines

Live, attenuated parainfluenza virus vaccines have been developed from both human and bovine strains for delivery by the intranasal route. Candidate vaccines should be able to replicate and induce a protective immune response in young infants in the presence of maternally acquired antibody. Achieving an appropriate balance between attenuation and immunogenicity has, however, been a major obstacle to the development of such vaccines.

Two attenuated strains have been studied: one is a *ts* human PIV-3 strain, *cp*45, which was selected after 45 passages of the virus in African green monkey cells at low temperature; the other is a bovine PIV-3 strain, which is closely related antigenically to human PIV-3, can protect monkeys against challenge with human PIV-3, and replicates poorly in humans. Both *cp*45 and bovine PIV-3 have been evaluated in Phase I/II trials in RSV seropositive and seronegative children and in young infants. Both candidates were found to be over-attenuated in seropositive children, but immunogenic in seronegative children and infants, although the magnitude of the anti-HN response was lower in children who received the bovine PIV-3 vaccine [69].

This prompted the engineering of chimeric bovine/human PIV-3 candidate vaccines that contain the human PIV-3 F and HN genes and internal genes from bovine PIV-3. One of such chimeric viruses, hPIV-3-Nb, is a human PIV-3 with the human nucleocapsid (N) gene replaced by its bovine counterpart. The virus was found to be attenuated and protective in non-human primates, and is at Phase I clinical trial stage.

Chimeric bovine PIV-3 expressing the F and HN proteins of human PIV-3 have been used as a backbone into which the F, or F and G ORFs of RSV A or RSV B were inserted to provide a bivalent candidate vaccine against RSV and PIV-3 infections in young infants [70].

A few attempts also have been made at developing PIV-1 vaccines. Sendai virus, a murine PIV-1, was found to protect African green monkeys against human PIV-1 challenge but does not seem to be sufficiently attenuated to be used as a Jennerian vaccine in human infants [71]. Attenuated chimeric viruses that contain PIV-3 *cp*45 internal genes with the F and HN genes from either PIV-1 or PIV-2 have been tested in hamsters [72]. In addition, Berna Biotech is developing a virosomal formulation of a PIV-3 vaccine.

5. Respiratory syncytial virus (RSV)

5.1. Disease burden

RSV is the most important cause of severe LRIs in infants and young children worldwide [67,69]. RSV disease spectrum includes a wide array of respiratory diseases, ranging from rhinitis and otitis media to pneumonia and bronchiolitis; the latter two diseases being associated with substantial morbidity and mortality. Humans are the only known reservoir for RSV. Spread of the virus from contaminated nasal secretions occurs via large respiratory droplets, so close contact with an infected individual or contaminated surface is required for transmission. RSV can persist for several hours on toys or other objects, which explains the high rate of nosocomial RSV infections, particularly in pediatric wards.

In temperate climates, RSV is well documented as a cause of yearly winter epidemics of acute LRI, including bronchiolitis and pneumonia. In industrialized countries, nearly all children by 2 years of age have been infected with RSV, which is estimated to be responsible for 18,000–75,000 hospitalizations and 90–1900 deaths annually in the USA. The incidence rate of RSV-associated LRI in otherwise healthy children was calculated as 37 per 1000 child-year in the first two years of life (45 per 1000 child-year in infants less than 6 months old) and the risk of hospitalization as 6 per 1000 child-year (11 per 1000 child-year in the first six months of life). Incidence is higher in children with cardiopulmonary disease and in pre-term infants, who constitute almost half of RSV-related hospital admissions in the USA. In the UK, it has been estimated that 74.8% of "unspecified bronchiolitis" and 16.3% of "unspecified pneumonia" admissions were RSV-related. The total annual incidence of hospital admissions attributable to RSV in this country was 28.3/1000 children less than 1 year of age and 1.3/1000 children 1–4 years old [73]. From figures available in industrialized countries, a global annual infection figure for RSV can be estimated around 64 million and mortality could be as high as 160,000.

Children who experience a more severe LRI caused by RSV have an increased incidence of wheezing and asthma later in life. These studies serve as a basis for anticipating widespread use of RSV vaccines in industrialized countries, where the costs of caring for patients with severe LRI and their

sequelae are substantial. RSV also is increasingly recognized as an important cause of morbidity from influenza-like illness in the elderly [74].

Few population-based estimates of the incidence of RSV disease in developing countries are available, although existing data clearly indicate that, there also, the virus accounts for a high proportion of LRIs in children. Studies in Colombia, Brazil, India and Thailand show that RSV causes 20–30% of LRI cases in children from 1 to 4 years of age, a proportion similar to that observed in industrialized countries. In addition to accurate incidence rates, other important data for developing countries are lacking, such as the severity and case-fatality rates for RSV infection at the community level and the median age of first infection. Preliminary data from community-based studies suggest that the median age of first infection may vary between communities. This information is important for vaccination program planners, when considering the optimal schedule for vaccination. For example, maternal immunization against RSV would be a desirable strategy to adopt if rates of infection during the first two months of life were found to be high.

Another confusing aspect of the epidemiology of RSV infection that may have an impact on vaccine use is the seasonality of the disease. In Europe and North America, RSV disease occurs as well-defined seasonal outbreaks during the winter and spring months. Studies in developing countries with temperate climates, such as Argentina and Pakistan, have shown a similar seasonal pattern. On the other hand, studies in tropical countries often have reported an increase in RSV in the rainy season but this has not been a constant finding. Indeed, marked differences in the seasonal occurrence of RSV disease have been reported from geographically contiguous regions, e.g. Mozambique and South Africa, or Bangladesh and India. Cultural and behavioral patterns in the community might affect the acquisition and spread of RSV infection. A clear understanding of the local epidemiology of the disease will be critical for the implementation of a successful vaccine development and introduction program [75–77].

5.2. Virology

RSV belongs to the family *Paramyxoviridae*, subfamily *Pneumovirinae*, genus Pneumovirus. The genome of RSV is a 15,222 nucleotide-long, single-stranded, negative-sense RNA molecule whose tight association with the viral N protein forms a nucleocapsid wrapped inside the viral envelope. The latter contains virally encoded F, G and SH glycoproteins. The F and G glycoproteins are the only two components that induce RSV neutralizing antibody and therefore are of prime importance for vaccine development. The sequence of the F protein, which is responsible for fusion of the virus envelope with the target cell membrane (see Section 3.2), is highly conserved among RSV isolates. In contrast, that of the G protein, which is responsible for virus attachment, is relatively variable: two groups of RSV strains have been described, the A and B groups, based on differences in the antigenicity of the G glycoprotein. Current efforts are directed towards the development of a vaccine that will incorporate strains in both groups, or will be directed against the F protein.

Promising inhibitors of the RSV fusion protein were abandoned partly because of resistant mutations that mapped to the F gene, reminiscent of the phenomenon observed with the peptide fusion inhibitors of HIV. A recent report that individual as well as dual infections with RSV and PIV could be prevented by short interfering RNAs (siRNAs) instilled intranasally in the mouse might pave the way to the development of a new strategy for anti-RSV and anti-PIV therapy in humans [78].

5.3. Vaccines

Development of vaccines to prevent RSV infection has been complicated by the fact that host immune responses appear to play a role in the pathogenesis of the disease. Early studies in the 1960s showed that children vaccinated with a formalin-inactivated RSV vaccine suffered from more severe disease on subsequent exposure to the virus as compared to unvaccinated controls. These early trials resulted in the hospitalization of 80% of vaccinees and two deaths. The enhanced severity of disease has been reproduced in animal models and is thought to result from inadequate levels of serum neutralizing antibodies, lack of local immunity, immune complex deposition and excessive induction of a type 2 helper T-cell-like (Th2) immune response with pulmonary eosinophilia and increased production of IL-4 and IL-5 cytokines [69,79].

In addition, naturally acquired immunity to RSV is neither complete nor durable and recurrent infections occur frequently. In a study performed in Houston, Texas, it was found that 83% of the children who acquired RSV infection during their first year of life, were reinfected during their second year, and 46% were reinfected during their third year [80]. At least two-thirds of these children also were infected with PIV-3 in their first two years of life. Older children and adults, however, usually are protected against RSV-related LRIs, suggesting that protection against severe disease develops after several successive infections.

Passive immunization with RSV-neutralizing immune globulins or humanized monoclonal antibodies given prophylactically has been shown to prevent RSV infection in newborns with underlying cardiopulmonary disease [81], particularly small, premature infants. This demonstrates that humoral antibody plays a major role in protection against disease. In general, secretory IgAs and serum antibodies appear to protect against infection of the upper and lower respiratory tracts, respectively, while T-cell immunity targeted to internal viral proteins appears to terminate viral infections.

5.3.1. Subunit vaccines

Although live attenuated vaccines seem preferable for immunization of naive infants than inactivated or subunit vaccines, the latter may be useful for immunization of the elderly

and high-risk children, as well as for maternal immunization. Candidate subunit vaccines based on purified F protein (PFP-1, -2 and -3) have been found safe and immunogenic in healthy adults and in children over 12 months with or without underlying pulmonary disease, as well as in elderly subjects and in pregnant women. A Phase I study of PFP-2 was conducted in 35 women in the 30–40th week of pregnancy: the vaccine was well tolerated and induced RSV anti-F antibody titers that were persistently four-fold higher in newborns to vaccinated mothers than to those who had received a placebo. No increase in the frequency or morbidity of respiratory disease was observed in infants from vaccinated mothers. Maternal immunization using a PFP subunit vaccine would be an interesting strategy to protect infants younger than 6 months of age who respond poorly to vaccination [69].

The efficacy of a subunit PFP-3 vaccine was tested in a Phase III trial on 298 children 1–12 years of age with cystic fibrosis. The vaccine was well tolerated and induced a four-fold increase in RSV neutralizing antibody titers, but this was not associated with significant protection against LRI episodes as compared to placebo recipients [82].

A subunit vaccine developed by Sanofi-Pasteur and consisting of co-purified F, G, and M proteins from RSV A has been tested in healthy adult volunteers in the presence of either alum or polyphosphazene (PCPP) as an adjuvant. Neutralizing antibody responses to RSV A and RSV B were detected in 76–93% of the vaccinees, but titers waned after 1 year, suggesting that annual immunization with this vaccine will be necessary.

A subunit approach also was investigated using the conserved central domain of the G protein of an RSV-A strain, whose sequence is relatively conserved among groups A and B viruses. A recombinant vaccine candidate, BBG2Na, was developed by fusing this domain (G2Na) to the albumin-binding region (BB) of streptococcal protein G (Pierre Fabre). The candidate vaccine elicited a protective immune response in animals, but was moderately immunogenic in adult human volunteers and its clinical development was interrupted due to the appearance of unexpected side effects (purpura) in a few immunized volunteers.

Another RSV candidate vaccine is a synthetic peptide of the conserved region of the G protein administered intranasally, either alone or in combination with cholera toxin. Protection was conferred to mice even without the cholera toxin.

5.3.2. *Live attenuated vaccines*

Live, attenuated RSV vaccines that could be delivered to the respiratory mucosa through intranasal immunization have been in development for more than a decade, based on temperature-sensitive (*ts*), cold-adapted (*ca*) or cold-passaged (*cp*) mutant strains of the virus. Difficulties for such a vaccine arise from over- or under-attenuation of the virus and limited genetic stability. Most attenuated live RSV strains tested in humans to date including *cpts* mutants that were attenuated in adults and seropositive children, caused mild to moderate congestion in the upper respiratory tract of seronegative infants 1–2 months old and, therefore, were considered as insufficiently attenuated for early infancy [83]. Recombinant RSV vaccines with deletion mutations in nonessential genes (SH, NS1 or NS2), and both *cp* and *ts* mutations in essential genes, are currently being evaluated.

Recombinant DNA technology also has provided the possibility of engineering a chimeric virus containing the genes of human PIV-3 surface glycoproteins F and NH, together with those of RSV glycoproteins F and G, in a bovine PIV-3 genetic background. A first candidate vaccine was found to be attenuated and to induce an immune response to both human PIV-3 and RSV in rhesus monkeys and will presently enter clinical trials [70]. Similarly, a bovine PIV-3 genome was engineered to express human PIV-3 F and HN proteins and either native or soluble RSV F protein [84]. Resulting recombinants induced RSV neutralizing antibodies and protective immunity against RSV challenge in African Green monkeys. These b/h PIV3/RSV F vaccines will presently be tested for safety and efficacy in human clinical trials as bivalent vaccines to protect infants from both RSV and PIV-3 infection and disease.

Finally, a combination of a live-attenuated vaccine with a subunit vaccine also is being considered for protecting adults against RSV illness, although a preliminary test of this strategy in healthy young and elderly adults was inconclusive.

6. Severe acute respiratory syndrome (SARS)

6.1. *Disease burden*

Severe acute respiratory syndrome (SARS) is a severe respiratory illness caused by a newly identified virus, the SARS coronavirus (SARS-CoV) [6,7]. The disease emerged in southern China in late 2002 and spread in the spring of 2003 to some 30 countries within Asia, Europe and North America. The epidemic finally came to a stop in July 2003 through strict implementation of quarantine and isolation procedures and international collaboration under the coordination of WHO. SARS is characterized by fever, headache, cough and dyspnea, and rapidly progresses to respiratory distress syndrome in more than 20% of the patients, who then necessitate prolonged hospitalization, intensive care and mechanical ventilation. The multiphasic course of the infection with recurrence of fever and disease after apparent initial improvement and a consistent CD4+ and CD8+ T-cell lymphopenia are reminiscent of the fatal multiphasic disease progression and acute T-cell lymphopenia observed in cats infected with the feline infectious peritonitis virus (FIPV), another coronavirus [85].

According to WHO, 8437 cases of SARS had been identified worldwide as of July 2003 and 813 patients had died, a 9.6% mortality rate. Only sporadic mini-outbreaks have been reported since then in China, Singapore and Taiwan,

two of which were linked to laboratory contaminations. Unapparent, nonpneumonic infections also seem to be quite common, as judged from seroprevalence in healthy populations of blood donors or medical personnel in Hong Kong [86]. Transmission is thought to mostly occur by respiratory droplets. Several instances of nosocomial infection have been reported and health care workers are at a high risk of infection.

Although there is evidence that SARS-CoV emerged from a non-human source, no animal reservoir has yet been identified with certainty. Masked palm civet cats and raccoon dogs have been found to be carriers of the virus, and Chinese wild animal traders show high seroprevalence figures, especially civet cats traders. SARS-CoV also has been recovered from rats but there is no evidence that it is naturally transmitted among that species. The virus has been found to multiply asymptomatically in mice and cats and is mildly pathogenic for some monkeys, ferrets and masked palm civets [87]. In spite of the limited extension and relatively rapid control of the epidemic by National Authorities and WHO, the highly contagious nature of the disease and its high fatality rate have prompted the search for a vaccine [88].

6.2. Virology

SARS-CoV belongs to a newly identified group in the family *Coronaviridae*, which are enveloped viruses whose envelope is characterized by crown-like proteinic spikes. Its RNA genome is an exceptionally long 29,727 nucleotides single-stranded positive RNA molecule which contains 11 open reading frames and encodes the replicase molecule (proteins 1a and b), spike protein (S), envelope protein (E), membrane protein (M), and nucleocapsid protein (N). Among other members of the *Coronaviridae* family are human coronaviruses HCoV-229E and HCoV-OC43 (agents of the common cold), the feline infectious peritonitis virus, the avian infectious bronchitis virus (IBV) and the pig transmissible gastroenteritis virus (TGEV). Two novel human coronaviruses, HCoV-HKU1 and –NL63, have recently been isolated from cases of pneumonia in humans [8–10].

The S protein of these viruses is known to be responsible for the induction of virus neutralizing antibodies. In some CoV species, the S protein is cleaved by a protease to yield two noncovalently associated subunits, S1 and S2: S1 contains the receptor-binding site while S2 forms the membrane-anchored stalk region and mediates the fusion between the viral and cellular membranes, as observed with influenzavirus HA, paramyxovirus F or HIV Env glycoproteins. Available data suggest, however, that the SARS-CoV spike glycoprotein S is not cleaved into two moieties, but contains the viral receptor-binding site and neutralization epitopes in its N-terminal half and a fusion domain and two heptad repeats, as well as another neutralization epitope in its C-terminal half [89–92].

The angiotensin-converting enzyme 2 (ACE2) receptor has recently been identified as a receptor for SARS-CoV [93].

6.3. Vaccine development

The S protein of SARS-CoV is a prime target for the generation of neutralizing antibodies against SARS-CoV and the development of protective humoral immunity. A human neutralizing monoclonal antibody targeted to the S protein was found to block the attachment of the virus to its receptor and provided remarkable protection in a mouse model of SARS-CoV infection, paving the way for its eventual use in passive serotherapy to provide immediate protection against infection for contacts and medical personnel.

Less than 1 year after SARS first appeared a half-dozen candidate vaccines were already in development. At this time, a number of candidate vaccines are on track:

- Several whole inactivated virus vaccine preparations, one of which already was tested in Phase I clinical trials in China.
- A live MVA recombinant vaccine and a live bovine PIV-3 recombinant vaccine, both expressing the SARS-CoV S protein.
- A live recombinant nonreplicative adenovirus vaccine expressing the S, M and N proteins.
- DNA vaccines expressing either the N or the S proteins.
- Several subunit vaccines made of recombinant SARS virus proteins.
- In addition, coexpression of SARS-CoV S, M, and N proteins in human 293 renal epithelial cells in culture resulted in the production of SARS-CoV VLPs that will eventually be developed into a particulate recombinant vaccine.

A recombinant SARS-CoV S glycoprotein/MVA vaccine was found to elicit a potent neutralizing antibody response after two immunizations in rhesus macaques and protected the vaccinated monkeys against virulent SARS-CoV challenge by the intranasal route [90].

All these vaccines face uncertainties, however, not the least of which is the lack of a reliable animal model in which to test them. Undertaking clinical trials of a SARS vaccine in a setting of vanishing perception of the potential risk of re-emergence of the disease is yet another difficulty. A final uncertainty is the possibility of immune enhancement, a phenomenon which was observed when studying vaccination of cats against the feline coronavirus, FIPV: animals vaccinated with a whole inactivated virus vaccine showed accelerated disease and death after exposure to wild type virus. The humoral response to FIPV does not seem to be protective but can in fact lead to drastically accelerated disease, presumably due to antibody-dependent enhancement of target cell infection via Fc receptor-mediated attachment of virus–antibody complexes. Control of infection and FIPV clearance are thought to primarily depend on cell-mediated immune responses [85].

The fact that ferrets vaccinated with an experimental MVA/SARS-CoV recombinant vaccine suffered from strong inflammatory responses in liver tissue upon SARS virus challenge [94] casts a cautionary note that immunization with some SARS vaccines might worsen the disease rather than prevent it.

7. Streptococcus pneumoniae

7.1. Disease burden

Based on available data, *S. pneumoniae* is estimated to cause more than one-third [95] of the 2 million annual child deaths following ARI [96], especially in developing countries where the bacterium is one of the most important bacterial pathogens of infancy and early childhood.

Virtually every child is colonized with one or more strains of *S. pneumoniae* and becomes a nasopharyngeal carrier during the first years of life. Carriage is more common and occurs at a younger age in children in developing countries. Many children go on to develop one or more episodes of otitis media, whereas a smaller number develop more serious invasive pneumococcal infections. Bacteremic pneumonia is a common form of invasive pneumococcal disease, the next most common being pneumococcal meningitis. Deaths from pneumonia far outnumber deaths from meningitis worldwide. In the USA, most cases of invasive pneumococcal disease are characterized by febrile bacteremia without specific localization. Incidence of invasive pneumococcal disease in less than 2-year-old infants in Europe ranges from 14 cases per 100,000 in Germany and The Netherlands to more than 90/100,000 in Spain [97]. Less severe but more frequent forms of pneumococcal disease include middle-ear infection, sinusitis or recurrent bronchitis. Thus, in the USA alone, 7 million cases of otitis media are attributed to pneumococci each year.

Although all age groups may be affected, the highest rate of pneumococcal disease occurs in young children and in the elderly population. In addition, persons suffering from a wide range of chronic conditions and immune deficiencies are at increased risk. In Europe and the USA, pneumococcal pneumonia accounts for at least 30% of all cases of community-acquired pneumonia admitted to the hospital, with a reported annual incidence of 5500–9200 per 100,000 persons 65 years of age or older, and a case fatality rate of 10% to 30%. There is, however, almost no data on the rates of pneumococcal disease in the elderly populations in developing countries. *S. pneumoniae* is an under-appreciated cause of nosocomial pneumonia in hospital wards, intensive care units, as well as in nursing homes and long-term care institutions [98].

S. pneumoniae also is the leading cause of non-epidemic childhood meningitis in Africa and other regions of the developing world. It has been estimated that up to 75% of children who develop pneumococcal meningitis either die or remain permanently disabled. Children infected with HIV/AIDS are 20–40 times more likely to contract pneumococcal disease than HIV uninfected children.

7.2. Bacteriology

S. pneumoniae is a Gram-positive encapsulated diplococcus. Based on differences in the composition of the polysaccharide capsule, around 90 serotypes have been identified. This capsule is an essential virulence factor. The majority of pneumococcal disease in infants is associated with a small number of these serotypes, which may vary by region and over time. Current data suggest that the 11 most common serotypes cause at least 75% of invasive disease in all regions. Several other virulence factors have been described, including pneumolysin, which leads to pore formation and osmotic lysis of epithelial cells, autolysin, and pneumococcal surface protein A (PspA), which interferes with phagocytosis and immune function in the host.

Pneumococci are transmitted by direct contact with respiratory secretions from patients and healthy carriers. Although transient nasopharyngeal colonization rather than disease is the normal outcome of exposure to pneumococci, bacterial spread to the sinuses or the middle ear, or bacteremia following penetration of the mucosal layer may occur in persons susceptible to the involved serotype, with secondary infection in distant sites, e.g. meningitis. Pneumococcal resistance to essential anti-microbials such as penicillins, cephalosporins and macrolides is a serious and rapidly increasing problem worldwide.

7.3. Vaccines

7.3.1. Polysaccharide vaccines

Protective immunity is conferred by type-specific, anticapsular antibodies, although the serological correlates of immunity are poorly defined. Antibodies to several pneumococcal surface proteins, including PspA, have been demonstrated to confer protection in animal models but the role of these antibodies in humans is yet to be determined.

Currently licensed vaccines are polyvalent polysaccharide vaccines containing per dose 25 μg of purified capsular PS from each of the 23 serotypes of *S. pneumoniae* that together account for most cases (90%) of serious pneumococcal disease in Western industrialized countries. Relatively good antibody responses (60–70%) are elicited in most healthy adults within 2–3 weeks following a single intramuscular or subcutaneous immunization [98]. The immune response is, however, mediocre in children aged less than 2 years, and in immunocompromised individuals (HIV/AIDS). Furthermore, PS vaccines do not induce immunological memory, which is required for subsequent booster responses. The polyvalent PS vaccine is recommended for healthy people over 65 years of age, particularly those living in institutions. Randomized controlled trials in healthy elderly people in industrialized countries have, however, failed to show a ben-

eficial effect of the vaccine, so that recommendation for its use in the elderly is based on data from observational studies showing a significant protective effect against invasive (bacteremic) pneumococcal disease, but not pneumonia.

Following the vaccination of pregnant women with PS vaccines, anti-PS antibodies are transferred both via the placenta and in the breast milk, but formal demonstration that maternal vaccination actually protects newborn infants against pneumococcal disease still is lacking [99].

7.3.2. Conjugate vaccines

Over the past 15 years, several vaccine manufacturers have developed pneumococcal conjugate vaccines in which a number of *S. pneumoniae* PS are covalently coupled to a protein carrier. Conjugate vaccines elicit higher antibody levels and a more efficient immune response in infants, young children, and immunodeficient persons than the PS vaccines, as well as a significant immunological memory resulting in a booster antibody response on subsequent exposure to the antigen. Moreover, these vaccines suppress nasopharyngeal carriage of the pathogen and reduce bacterial transmission in the community leading to herd immunity, which adds considerable value to their implementation. Conjugate vaccines immunization followed by PS vaccine boosting might provide a foundation for life-long protection against pneumococcal disease.

In a double-blind Phase III study of the 7-valent vaccine, Prevnar (Wyeth), conducted at Northern California Kaiser Permanente medical centers on 37,868 infants, 40 cases of invasive *S. pneumoniae* disease were seen in the study population, 39 of which were in the control group, representing a 97% vaccine efficacy. The vaccine was found to be 100% efficacious in the few low birth-weight and pre-term infants included in the study. Another efficacy trial of the 7-valent conjugate vaccine in the USA in the native American population showed efficacy of 83% (intent-to-treat) against invasive disease. The vaccine also showed 20–30% reduction in radiologically confirmed pneumonia in the studies in California. [100,101].

Two different 7-valent conjugate vaccine formulations elicited moderate protection against otitis media, as the decrease in cases of vaccine-type otitis media was offset by an increase in those due to non-vaccine-types of *S. pneumoniae* and by *H. influenzae*, a phenomenon referred to as "replacement disease". However, vaccination did result in a significant reduction in otitis media requiring tympanostomy tube placement [102].

A trial to measure the effect on otitis media of an 11-valent vaccine with *H. influenzae* protein D as carrier (GSK) is ongoing in the Czech Republic. This vaccine may provide additional protection against *H. influenzae* or limit replacement due to this organism. Results of the trial are expected shortly.

Introduction of the conjugate vaccine in early 2000 in the USA resulted in dramatic decline in the rates of invasive pneumococcal disease, with significant reductions also been seen in unvaccinated individuals as a result of herd immunity [103,104]. Introduction of the vaccine was also shown to reduce disparity in disease rates between black and white children [105]. Though the per cent reduction of disease in the unvaccinated children and adults were lower than in vaccinated children, the number of cases of invasive disease prevented through the indirect effects of the vaccine are estimated to be greater than those prevented through the direct effect. Significant increase in rates of invasive disease due to non-vaccine serotypes of pneumococcus were observed following vaccine introduction in the USA. However, the increase was small relative to the decrease in vaccine-type invasive disease caused by vaccination.

The currently licensed 7-valent vaccine, Prevnar, does not contain some of the serotypes that cause severe disease in developing countries, notably serotypes 1 and 5. New conjugate vaccines containing 9–11 serotypes that provide more optimal serotype coverage in these countries are in clinical development, including a 9-valent vaccine (Wyeth), and two 11-valent vaccines (GSK and Sanofi-Pasteur).

Trials have been conducted in two African countries using the 9-valent conjugate vaccine that contains serotypes 1 and 5 in addition to those in the 7-valent vaccine. The first study is South Africa, involving 40,000 subjects, showed efficacy (intent-to-treat) of 83 and 65% against vaccine type invasive disease in HIV-infected and -uninfected children, respectively. Interestingly, the study also showed a 31% reduction in virus-associated pneumonia (influenzavirus or paramyxoviruses) requiring hospitalization, suggesting that dual infection with viruses and pneumococcus are common among children with severe disease requiring hospitalization [14]. The recently published results of the trial in The Gambia showed 77% efficacy against vaccine type invasive disease, and 37% efficacy against radiological pneumonia. Very significantly, this study also showed 16% reduction in mortality in the vaccinated group [95].

An 11-valent vaccine with tetanus and diphtheria toxoids as the protein carrier (Sanofi-Pasteur) is undergoing an efficacy trial in the Philippines, but this vaccine is unlikely to be developed up to licensure. In addition, an improved formulation of the GSK 11-valent conjugate vaccine is also in clinical trials.

7.3.3. Protein subunit vaccines

Newer vaccine approaches also are being developed in order to provide protective immunity against a larger number of *S. pneumoniae* serotypes, and to circumvent the complexity of manufacture of conjugate vaccines [106]. Several pneumococcal proteins, including pneumolysin, PspA, pneumococcal surface adhesin (PsaA), neuraminidase, and autolysin are at an early clinical stage development. PiaA and PiuA, two newly identified lipoprotein components of *S. pneumoniae* iron uptake ABC transporters, elicit protective immunity against invasive pneumococcal disease in mice through induction of opsonophagocytosis-promoting antibodies. PspA has been tested in Phase I trials and was shown

to elicit antibodies that protected mice from otherwise fatal pneumococcal infection [107].

Through screening of a *S. pneumoniae* genomic expression library with human convalescent sera, Shire Biologicals, Canada (now ID BioMedical) has identified what appear to be remarkably conserved bacterial surface proteins (BVH-3 and BVH-11) that are able to induce protective anti-pneumococcal antibodies in the mouse model. A recombinant 100 kD hybrid protein, BVH3/11V, was engineered by fusion of parts of the two genes and expressed with high yields in *E. coli*. The fusion protein has successfully been tested in Phase I dose ranging clinical trials in toddlers and elderly volunteers, and Phase II clinical studies in infants and elderly persons have been initiated (personal communication). This vaccine should be serotype-independent as the BVH3 and BVH11 antigens are common to all 90 serotypes of *S. pneumoniae*.

8. Concluding remarks

Acute respiratory infections remain the most common cause of morbidity and mortality in children worldwide. They also constitute an important cause of mortality in the elderly population. Emerging infections such as SARS and the lurking risk of pandemic influenza pose new threats. New developments in the field of vaccinology offer a real possibility of controlling this problem through immunization. Since infections with multiple pathogens appears to contribute to the severity of disease, the prevention of one infection offers the possibility of morbidity reduction from other respiratory pathogens as was demonstrated in the case of pneumococcal vaccine and viral respiratory infections. However, there are a number of obstacles that still need to be overcome. Available effective vaccines are limited in supply and only available at a high price, putting them out of the reach of the most vulnerable populations in developing countries. Several other vaccines face technical challenges or obstacles to development related to intellectual property rights. Considering the serious problems that acute respiratory infections pose globally, there is an urgent need for a concerted effort to overcome these obstacles and make these life saving vaccines available and affordable to all the citizens of the world.

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